TRANSLATION

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference LeA30218PCBu	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 95/02358	International filing date (day/month /year) 19.06.1995	Priority date (day/month/year) 01.07.1994
International Patent Classification (IPC) or national classification and IPC C07K14/54		
Applicant BAYER AKTIENGESELLSCHAFT et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of the invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability: citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 05.10.1995	Date of completion of this report 07.06.1996
Name and mailing address of the IPEA/ EP Facsimile No.	Authorized officer Telephone No.

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

national application No.
PCT/EP95/02358

I. Basis of the report

1. This report has been drawn on the basis of (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

[x] the international application as originally filed.

[ ] the description. pages _____ . as originally filed.
pages _____ . filed with the demand.
pages _____ . filed with the letter of _____ .
pages _____ . filed with the letter of _____ .

[ ] the claims. Nos. _____ . as originally filed.
Nos. _____ . as amended under Article 19.
Nos. _____ . filed with the demand.
Nos. _____ . filed with the letter of _____ .
Nos. _____ . filed with the letter of _____ .

[ ] the drawings. sheets/fig _____ . as originally filed.
sheets/fig _____ . filed with the demand.
sheets/fig _____ . filed with the letter of _____ .
sheets/fig _____ . filed with the letter of _____ .

2. The amendments have resulted in the cancellation of:

[ ] the description. pages _____
[ ] the claims. Nos. _____
[ ] the drawings. sheets/fig _____

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

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V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1, 2 in part	YES
	Claims	1, 2 in part	NO
Inventive step (IS)	Claims		YES
	Claims	1, 2	NO
Industrial applicability (IA)	Claims	1, 2	YES
	Claims		NO

2. Citations and explanations

1. The following search report citations are considered in this international preliminary examination report:
D1 to D5;
the same numbering will also be used in the same sequence in the subsequent proceedings.

2. The present application does not satisfy the criterion of PCT Article 33(2) since the subjects of claims 1 and 2 are not novel in the light of the prior art as defined in the Regulations (PCT Rule 64.1 to 64.3).

D1 (claims 2 to 5) claims therapeutic agents which are antagonists or partial agonists of hIL-4 or contain the latter, are hIL-4-mutant proteins and, in addition to the amino acid replacements in positions 121, 124 and/or 125, have the ability to break off the polypeptide chain, so satisfying the claimed criterion for modification at the C-terminal end of the polypeptide chain.

3. The present application does not satisfy the criterion of PCT Article 33(3) since the subjects of claims 1 and 2 do not involve an inventive step (PCT Rule 65.1, 65.2).

The technical problem addressed by the present application is to provide novel human interleukin-4-mutant proteins which are effective as antagonists or partial agonists of human interleukin-4 (hIL4), and additionally have greater stability and can be purified more easily.

The above technical problem is solved in that, in addition to replacing amino acids 121, 124 or 125 in the hIL-4 chain, additional modifications occur at the N and/or C-terminal, and/or potential glycosylation sites are deleted and/or the mutant protein is coupled to a non-protein polymer.

D1, the closest prior art, discloses hIL-4-mutant proteins which were each subjected to amino acid replacement in positions 121, 124 and 125 of the polypeptide chain. Antagonistic or partially agonistic effects of the muteins towards hIL-4 could thus be attained (cf. examples 1 to 3 of D1). The muteins described are used as medicaments.

D2 describes the importance of the inactivation of N-glycosylation sites in the hIL-4 molecule in order to overcome problems such as low yields in isolation and purification or the immunogenic effects of molecules containing sugar residues. Success is achieved here with the replacement in the hIL-4

molecule of amino acids which can potentially be used for glycosylation with amino acids which are structurally unsuitable for that purpose and thus alter the detection sequence **Asn-A^1-Z** (A^1 can be any amino acid; Z = Ser, Thr) for glycosylating enzymes such that *in vivo* glycosylation is no longer possible (cf. page 9, line 5 to page 12, line 2 and the claims).

D5 describes the conversion of hIL-4 into a high-affinity antagonist (IL-4 variant **Y124D**) by replacing **Tyr124** with **Asp**. The substitution of **Tyr124** by **Phe**, **His**, **Asn** or **Gly** leads to partial agonists with uninfluenced receptor-bonding affinity. The significance of the C or N-terminal for the spatial arrangement in the IL-4 molecule and thus for the biological activity (as antagonist or agonist) is mentioned on page 3241 (right-hand column, lines 20 to 43).

D3 describes the covalent bonding of non-protein polymers (polyethylene glycol) to recombinant hIL-4 for increasing the half-life value (cf. column 3, line 58 to column 4, line 32 and example 12).

Like D1, D4 discloses hIL-4 mutations which, owing to amino acid replacement in positions **Arg121**, **Tyr125** and **Ser125**, lead to antagonists or partial agonists of hIL-4.

The combination of the technical features of D1 and D2 to D5 is an obvious approach for a person skilled in the art wishing to solve the technical problem.

Replacing amino acids in positions 121, 124 and 125 leads to mutants having antagonistic or partially agonistic activity towards hIL-4. With the teaching of D5 concerning the importance of the C or N-terminal for the spatial structure, a person skilled in the art occupied with solving the technical problem will be prompted to modify the C/N-terminal ends of the mutated hIL-4 peptide chain. The same applies to the deletion of the glycoside-bonding sites (cf. D2). The conjugation of non-protein polymers in order to increase the stability of rhIL-4 is explicitly disclosed in D3 and is anticipated as a way of solving the problem of stability.

The present application does not indicate that the hIL-4 muteins have antagonistic or partially agonistic properties which, as an unforeseeable effect, would imply inventive activity.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. The application does not satisfy the requirements of PCT Article 6 since claim 1 is unclear. "Positions 121, 124 and 125" in claim 1 are not clearly defined as positions in the peptide chain or amino acid sequence of the hIL-4 muteins. The term "additional modifications" in claim 1 likewise contravenes the requirements of PCT Article 6 since it does not allow any conclusions to be drawn about the type of modification (however, see page 6, lines 28 to 30 of the application which shows the possible modifications as **deletions**, **insertions** or **substitutions**).